Stability Study of Multiwalled Carbon Nanotube/κ-Casein Dispersion

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Abstract

As one of the most promising materials, multiwalled carbon nanotubes (MWNTs) have been developed for bioapplications. However, the poor dispersion in either water or organic solvents has largely restricted the research and applications of MWNTs. In this study, MWNTs were homogeneously dispersed in aqueous solution ofκ-casein without chemical modification. Techniques including Zeta potential, transmission electron microscopy, circular dichroism have been employed to study the influencing factors on the stability of the dispersion. The results indicated that the dispersion of MWNTs in 0.05%wt κ-casein is the most stable when the concentration of MWNTs is 0.1%wt. The exposure of phenylalanine in κ-casein has a significant impact on the dispersion of MWNTs because of the π-π stacking and strong van der Waals attractions between the phenylalanine and MWNTs. The high stability of dispersion may develop a new and economic way to use this common protein for bioapplication and biomaterial in the future.

Keywords

MWNT Dispersion; κ -casein; Circular Dichroism; Phenylalanine

Introduction

Multiwalled carbon nanotubes (MWNT), unique nanomaterials with extraordinary mechanical and electronic properties, have attracted considerable interest(Lisa V, 1998). An ideal carbon nanotube consists of multiple rolled layers of graphite, derived from unusual carbon structure by metal oxidation catalyst (Iijima S, 1991). However, aqueous suspensions of MWNT are unstable due to the weak interaction between the solution and the MWNT (Kim J, 2008). Moreover, strong van der Waals attractions among MWNTs lead to fast precipitation in the system. For these reasons, applications of MWNT are largely limited in various aspects, especially in industrial scale (Krishna CE, 2010). Currently, two approaches are widely used in nanotube dispersion—the mechanical approach and the chemical approach (Dieckmann, GR. 2003; Boussaad S, 2003; Wang L 2003; Erlanger BF, 2001; Fu KF, 2002; Gooding JJ, 2003; Pantarotto D, 2003). The mechanical approach includes ultrasonication and high-shear mixing. These processes are time consuming and less efficient (Dieckmann, GR. 2003; Boussaad S, 2003). Moreover, this approach uses toxic and nonbiocompatible surfactants such as sodium dodecyl benzenesμlfonate, odecyltrimethylammonium bromide, hexadecyltrimethylammonium bromide, and octyl phenol ethoxylate (Triton X-100). Besides the toxicity of the surfactants, poor dispersion is another problem (Richa R, 2008; Islam MF, 2003; Whitsitt EA, 2003). On the other hand, the chemical approach improves the solubility by incorporating functional groups upon the surface of MWNTs. However, chemical functionalization produces defects on the surface of MWNT, which consequently alter the mechanical and electrical properties (Richa R, 2008; Islam MF, 2003; Whitsitt EA, 2003; Ryabenko AG, 2004; Wang H, 2003; Yu J, 2007).

Caseins, a family of phosphoproteins (αS1, αS2, β, κ) that account for nearly 80% of bovine milk proteins, form soluble aggregates because the κ-casein molecules in casein stabilize the micelle structure (Song F, 2010; Ismail MH, 2010; Bachtold A, 2001; Peigney A, 2003; Ajayan PM. 1999; Barone PW, 2005). The stability of the casein micelle is dependent on the presence of κ-casein on the surface of the micelle where it functions as interface between the hydrophobic caseins of the micelle interior and the aqueous environment. Since κ-casein forms micelles in aqueous solution and has better water solubility than α-casein and β-casein, it is expected that κ-casein can be used as the dispersant to create stable MWNT dispersion.

In this study, stable MWNT dispersions with pH-sensitive properties were prepared in aqueous κ-casein solution. The MWNT dispersion was characterized with transmission electron microscopy (TEM), and zeta potential. Ultraviolet circular dichroism (UV-CD) was used to determine the changes in secondary structure of κ-casein and the phenylalanine (Phe) of κ -casein exposed in the

solution. It is expected that the MWNT dispersion in κ-casein can be used in biomedical field, such as biosensors, functional surfaces and coating, biorecognition as well as drug delivery.

Experiment Section

Separation and Purification of κ-casein

5 g casein was dissolved at 200 ml 0.2M NaOH and heated to 55°C, and then 2.22 g CaCl2 was slowly added to the solution to precipitate α-casein and β-casein. After stirring for 30 min, pH was adjusted to neutralization, followed by centrifugation at 6000 rpm for 20 min. The coagulum was wiped off, while the supernatant was adjusted to pH 3.8 to precipitate the κ-casein. After the precipitate was dried by freeze drying at -40°C for 24 h, κ-casein was obtained (Zhang Y, 2009).

Preparation of MWNT Dispersion in Aqueous κ-Casein Solution

The MWNTs provided by Sichuan Nanotech (China) were obtained by chemical vapor deposition with a purity > 95%, and used without further purification. 0.1% MWNT was added to a solution containing 0.01%, 0.02%, 0.05%, 0.1%, 0.2%, or 0.5% of κ-casein at pH = 2, 7.4 and 12, respectively. All solutions were subjected to ultrasonication at room temperature and a nominal frequency of 50 kHz for 10 min.

Characterization

TEM was recorded using JEM100CX at an acceleration voltage of 80 kV. The dispersion was placed onto a copper grid, followed by drying at room temperature for 1 h. The UV-CD measurements were carried out with a 0.1-cm path length on a Jasco J-810 spectrophotometer. 100μl MWNT dispersion was put into a quartz cell and diluted to 1 ml using PBS (0.1 M) of the same pH value. All samples were scanned at 25°C with 3 times accumulation at 200 nm/min from 200 nm to 300 nm. Yang's equation (Yang AS, 1995) was used to calculate the ratio of secondary structure. Zeta potential measurements were carried out with a 1cm cell on a Zeta Potential Analyzer from Brookhaven Instruments Corporation. 100 μl MWNT dispersion was put into the cell and diluted to 1 ml by ultra-purified water. The experiments were repeated at least three times for averaging.

UV-vis spectrum was recorded using TU-1810 spectrophotometer in quartz cell with a path length of 1 cm.

Results and Discussion

Figure 1 shows the aqueous suspensions containing 0.1% MWNT stabilized with 0.05% κ-casein. The suspensions in Figures 1a and 1b are freshly prepared and stored for 30 days, respectively, with pH = 2, 7.4 and 11. It is easily observed from Figure 1a that there is no visible sedimentation for the three freshly prepared suspensions.

(a) (b)

FIG. 1 DISPERSION OF 0.1 WT% MWNT IN 0.05WT% AQUEOUS K-CASEION SOLUTION FRESHLY PREPARED (a) AND STORED FOR 30 DAYS (b) AT pH = 2.0, 7.4, 11.0 (FROM LEFT TO RIGHT)

For Figure 1(b), it is observed that suspension at pH = 2 exhibits precipitates and the other two suspensions at pH=7.4 and 11 remain homogeneous. The outstanding stability of the two suspensions can be attributed to increased physical interactions of the π-π stacking between nanotubes and phenylalanine (Phe) in the κ-casein. Therefore, Phe has a strong tendency to be absorbed on MWNT due to the π-π conjugated effect, which prevents re-aggregation and subsequent sedimentation of the nanotubes. However, the suspension is unstable at pH=2, because the chains of κ-casein have winded up and few Phe was exposed to interact with MWNTs in the suspensions.

In an effort to investigate the reason for the outstanding stability of MWNT dispersion caused by κ-casein, zeta potential has been measured and the experimental results are shown in Figure 2.

As shown in Figure 2, all the suspensions with different concentrations of κ -casein under different pH conditions exhibit a negative Zeta potential value due to the negative charge of MWNT. Among all the suspensions, the sample with a concentration of κ-casein of 0.05% under pH = 11 has the largest negative zeta potential value of -41.09 mV.

Riddick (Riddick TM, 1968) defined zeta potential with relation to the stability of dispersions as follows: (i) 0 to ±3 mV: maximum agglomeration and precipitation ; (ii) ±3 to ±5 mV: strong agglomeration and precipitation; (iii) ±10 to ±15 mV: threshold of agglomeration; (iv) ±16 to ±30 mV: threshold of delicate dispersion; (v) ±31 to ±40 mV: moderate stability; (vi) ±41 to ±60 mV: fairly good stability; (vii)

±61 to ±80 mV: very good stability and (viii) ±81 to ±100 mV: extremely good stability.

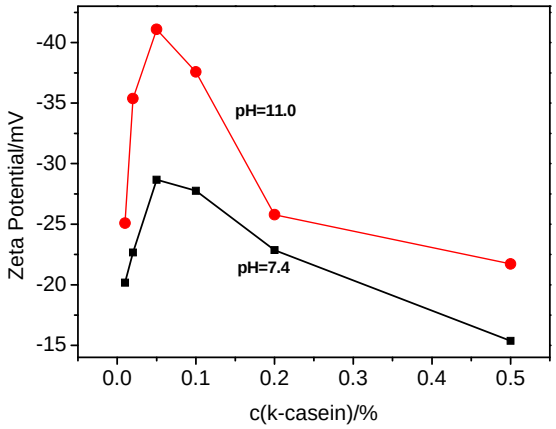


FIG. 2 CONCENTRATION EFFECT OF K-CASEIN ON THE ZETA POTENTIALS OF DISPERSIONS UNDER DIFFERENT pH CONDITIONS

According to Riddick's definition and the experimental results in Figure 2, it can be concluded that the suspension with 0.05% κ-casein is the most stable among all the suspensions and it has fairly good stability. Neither a low nor a high concentration of κ-casein exhibits the capability to disperse the MWNT in the suspension. This is because only limited quantities of micelles are formed when the concentration of κ-casein is very low in the suspension, which can not disperse nanotubes. As for the high concentration of κ-casein, the κ-casein will aggregate in the solution. Therefore an optimum concentration of κ-casein is necessary for the stability of the suspension.

pH is another factor affecting the zeta potential values. For pH=7, the zeta potential values of suspensions range from -15.36 mV to -28.37 mV. For pH=11, the range is from -21.72 mV to -41.09 mV. This is because the pH value affects the zeta potential value via changing the solubility of κ-casein. As for κ-casein, it is soluble and easily dispersed in the aqueous solution of 0.1 M PBS at pH=7.4, but the molecule chain is still fold (to be discussed in the following section). Hence, very limited amounts of phenylalanine in κ-casein are exposed to interact with MWNTs. While the molecule chain of κ-casein is unfold at pH=11, larger amounts of phenylalanine are exposed to interact with MWNTs, making the dispersion more stable.

Circular dichroism spectroscopy with a wavelength range of 200-300 nm was used to investigate the secondary structure of proteins. The far-UV CD spectrum within 200-250 nm of proteins can reveal the important characteristics of their secondary structure and estimate the conformations in the form of alpha-helix, beta-sheet, beta-turn or random coil (Sreerama N, 1999). The near-UV CD spectrum (250-300 nm) of proteins provides information on the tertiary structure, especially whether Phe has been exposed in the solution (Manavalan P, 1983).

The measurements were carried out under pH=2, 7, 11, and samples include pure aqueous κ-casein solution, κ-casein/MWNT dispersion, and κ-casein solution after centrifugation from the suspension. After the test, the Yang's equation was utilized to calculate the ratio of secondary structure (Yang AS, 1995). The results are shown in Figure 3.

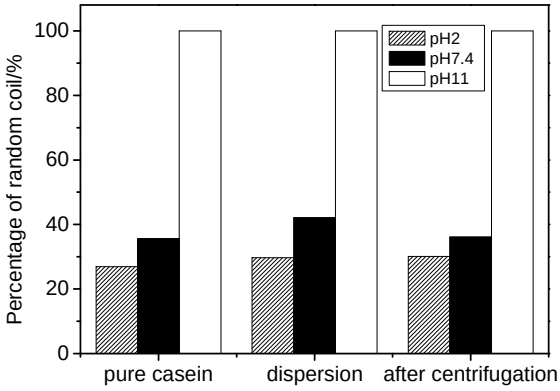


FIG. 3 PERCENTAGE OF RANDOM COIL OF K-CASEIN IN PURE AQUEOUS K-CASEIN SOLUTION, IN DISPERSION OF MWNT(0.1%) WITH K-CASEIN, IN K-CASEIN SOLUTION AFTER CENTRIFUGATION FROM THE DISPERSION AT pH = 2, 7.4, 11.

Figure 3 shows the content of random coil of κ-casein calculated by Yang's equation from CD spectrum. It is observed that the content of random coil increases with the change of pH value from acidic to alkaline environment, indicating that the conformation of κ-casein becomes more stretched while pH increases. Because κ-casein undergoes self-aggregation in acidic environment, the content of random coil will decrease due to aggregation. With the increment of pH value from acidic to neutral, κ-casein becomes stretched and soluble in PBS (pH = 7.4). When the pH value further increases from neutral to alkaline, the secondary structure of κ-casein becomes much more stretched, and the content of random coil increases even more. Though κ-casein may not be able to maintain its physiological activity at alkaline environment, the dispersion should be more stable because the

phenylalanine (Phe) in κ-casein will interact more strongly with MWNT when the chain is more stretched. Since the benzyl side chain in Phe can interact with MWNT by π-π stacking effect and van der Waals attractions, therefore the more Phe is exposed to the solution, the more stable the dispersion will be.

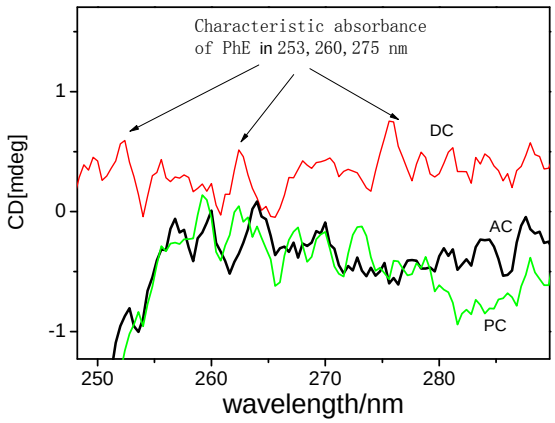


FIG. 4(a) CD SPECTRA OF K-CASEIN FOR PURE K-CASEIN SOLUTION(PC), K-CASEIN SOLUTION AFTER CENTRIFUGATION FROM THE DISPERSION (AC), DISPERSION OF MWNT(0.1%) IN AQUEOUS K-CASEIN SOLUTION(DC) AT pH = 7. K-CASEIN CONCENTRATION IS 0.05WT%

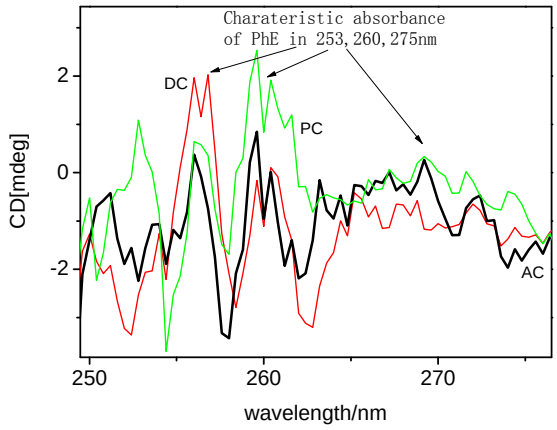
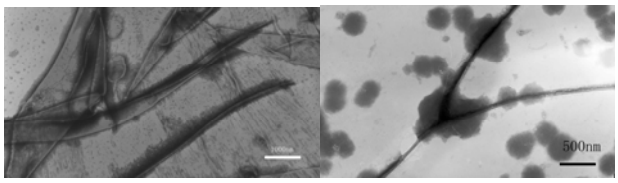


FIG. 4(b) CD SPECTRA OF K-CASEIN FOR PURE K-CASEIN SOLUTION(PC), K-CASEIN SOLUTION AFTER CENTRIFUGATION FROM THE DISPERSION (AC), DISPERSION OF MWNT(0.1%) IN AQUEOUS K-CASEIN SOLUTION(DC) AT pH = 11. K-CASEIN CONCENTRATION IS 0.05WT%

Figures 4 (a) and (b) show the CD absorbance of κ-casein at 253 nm, 260 nm, and 275 nm, which represents the exposure of phenylalanine (Phe) in the aqueous solution. The intensities at pH=11 are about twice than those at pH=7 with the same concentration of κ-casein (0.05%), indicating that the stability of pH=11 dispersion is better than that of pH=7 dispersion (Kelly SM, 1997; Price NC, 2000; Doekal M, 2000) and this is in agreement with the results of zeta potential measurement.

TEM was used to visualize nanotube microstructures in MWNT suspensions. Figure 5 shows the TEM pictures of 0.1% MWNT suspensions dispersed by 0.05% and 0.5% κ-casein solutions at pH=11. Dark round dots in Figure 5 are dyed κ-casein. As expected, it is observed that MWNTs in suspensions treated with 0.5 % κ-casein existed in a bundled state, as shown in Figure 5b. In contrast, the 0.05% κ-casein suspension contained more exfoliated MWNTs as shown in Figure 5a. This concentration-dependent MWNT microstructure in suspensions can be attributed to conformational transitions of the adsorbed κ-casein on the nanotube sidewalls that counteracts the inter-tube van der Waals attraction among MWNTs (Kim HS, 2009; Krishna C, 2010).



(a) (b)

FIG. 5 TEM PICTURES OF DISPERSION OF 0.1% MWNT IN 0.05% K-CASEIN (a) AND IN 0.5% K-CASEIN (b)

UV-vis spectrum was used to determine the concentration of MWNTs in the dispersion (dispersion efficiency). It was reported that CNTs show a characteristic absorption at 2.49 eV in XPS measurement (Wang D, 2005), which could be assigned to 500 nm in the UV-vis spectrum analysis. Therefore, absorption at 500 nm is used to determine the CNTs concentration in the dispersion. The dependence of absorbance on concentration at 500 nm is proved to obey the Beer's law.

Figure 6 shows the plot of dispersion efficiency and UV-vis absorption of MWNT-κ-casein dispersion versus κ-casein concentration at pH=11. It is regarded that 10 minutes sonication is enough to debundle all MWNTs that can potentially be debundled [17]. It is observed in figure 6 that the dispersion efficiency and UV-vis absorption are much lower when the κ-casein concentration is too low (0.01% and 0.02%) or too high (0.5%). When the concentration of κ-casein is too low, inadequate κ-casein interactions with MWNTs lead to self assembly of MWNTs to form precipitate in the

dispersion. When the concentration of κ -casein is too high, the κ-casein molecules tend to self assemble instead of forming stable micelles (Anneke H, 2006).

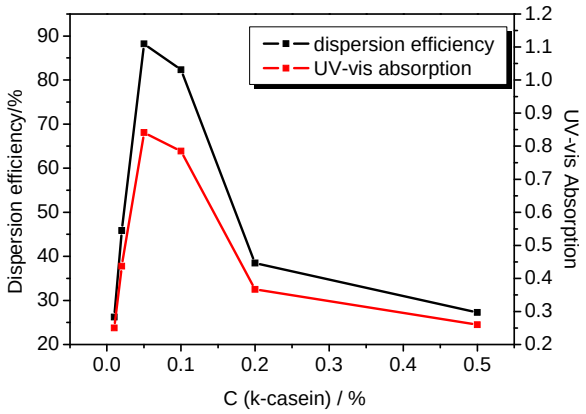


FIG. 6 PLOT OF DISPERSION EFFICIENCY, UV-VIS ABSORPTION OF MWNT K-CASEION DISPERSION VERSUS K-CASEIN CONCENTRATION AT pH = 11

Consequently, it is necessary to find out the optimum concentration for the κ-casein to disperse the MWNTs. In the UV-vis measurement, 500 nm was selected to investigate the dispersion efficiency of MWNTs. When the concentration of κ-casein is 0.05% or 0.1%, the dispersion efficiency of MWNTs is more than 80%. This result demonstrates that κ -casein is more efficient in dispersing MWNTs than other surfactant like SDS (Su JW, 2009; Yu J, 2007). It was reported that the SDS concentration should be at least 10 times higher than MWNT concentration in order to obtain a stable dispersion. The weight ratio of SDS to MWCNT is 0.1wt% .

Conclusions

Stable and pH-sensitive MWNT dispersions in κ -casein have been prepared without chemical modification. The dispersion remains stable under neutral or basic condition without sedimentation or gelation for more than 30 days. The π-π stacking interactions and strong van der Waals attractions between MWNT and phenylalanine in κ-casein play an important role in the stability of the suspension. Under neutral or basic condition, sufficient exposure of phenylalanine in κ-casein leads to a fairly good dispersion of MWNTs. An optimum concentration of κ-casein is necessary for the stability of the suspension. The high stability of dispersion may develop a new and economic way to use this common protein for bioapplication and biomaterial in the future.

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